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Changing Epidemiology of *Clostridium difficile*–Associated Disease during Stem Cell Transplantation

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A B S T R A C T

The incidence and severity of *Clostridium difficile*–associated disease (CDAD) within the general population has risen dramatically over the past decade, yet little data are available from hematopoietic stem cell transplantation (HSCT) centers. In the present study, we performed a chart review of 822 consecutive autologous and allogeneic HCST recipients treated at Northwestern Memorial Hospital between 2004 and 2008 to determine the incidence of CDAD at our institution. Variables including age, sex, diagnosis, chemotherapy regimen, transplantation type, microbial colonization, coinfections, diet, antibiotic use, neutropenic fever, comorbid conditions, time to engraftment, growth factor administration, and occurrence of graft-versus-host disease were assessed as potential risk factors for the development of CDAD. Eighty-five CDAD cases (10.3%) were identified. Bivariate analysis revealed a significant association between CDAD and neutropenic fever, administration of a neutropenic diet, ciprofloxacin and aztreonam use and duration of therapy, vancomycin and aztreonam use and duration of therapy, receipt of an allogeneic transplantation, bacterial coinfection, and vancomycin-resistant *Entereococcus faecium* (VRE) colonization. Cox regression analysis identified the following as factors associated with the development of CDAD: age >60 years, allogeneic transplantation, and prior VRE colonization. Allogeneic recipients with CDAD experienced increased higher rates of grades II to IV gastrointestinal graft-versus-host disease and nonrelapse mortality. A risk stratification model was developed to identify HSCT recipients at different levels of risk. With an incidence >10%, CDAD is a significant infectious complication of stem cell transplantation.

INTRODUCTION

Clostridium difficile–associated disease (CDAD) is linked to significant morbidity and mortality. An increase in the incidence and severity of CDAD has been observed both in the community and in hospitalized patients over the past 10 years, with outbreaks of a new hypervirulent CDAD (BI/NAP-1) strain increasingly reported across North America and Western Europe over the last 5 years. Currently, CDAD is the most common source of hospital-acquired diarrhea. Gastrointestinal (GI) surgery, antibiotic use, and medical comorbid states are known to increase the risk for developing CDAD in the general population [1-4].

The period surrounding hematopoietic stem cell transplantation (HSCT) is accompanied by profound changes within the GI tract. Prolonged neutropenia, exposure to prophylactic and empiric antimicrobials, damage to the GI tract from chemotherapy conditioning regimens, immunosuppression, microbial coinfection, suppression of gastric acid by H_2 blockers and proton pump inhibitors, reduced oral alimentation, and administration of low-microbial diets change the GI microenvironment, cause diarrhea in most HSCT recipients, and may predispose patients to develop CDAD [5,6]. Single-institution epidemiologic studies from HSCT centers have reported the incidence of CDAD between 5% and 18%; however, most of these studies were conducted more than a decade ago. Since then, transplantation practices have evolved (transplantation type, conditioning regimens), along with changes in the spectrum of infectious microorganisms and new antimicrobial agents used to fight infections, which might have an impact on the risk of developing CDAD.

We performed a chart review study in 822 consecutive autologous and allogeneic HSCT recipients treated at Northwestern Memorial Hospital (NMH) between 2004 and 2008 to determine the incidence of CDAD to analyze patient-, disease-, and transplantation-specific characteristics as potential risk factors associated with the development of CDAD and to report clinical outcomes. A risk stratification model was formulated to identify patients at the different levels of risk for developing CDAD.

METHODS

Electronic medical records for 822 consecutive HSCT recipients who underwent their transplantations between August 2004 and August 2008 were used to screen for CDAD. Pharmacy records were used to obtain data on antibiotic usage. All HSCT recipients underwent their transplantations as hospital inpatients. CDAD was defined as the occurrence of diarrhea and either a positive result from a laboratory stool assay for *C. difficile* toxin A or B or a positive culture from a toxigenic *C. difficile* strain. Both culture and toxin assay were performed in all patients with diarrhea tested for *C. difficile*.

Patients were screened for *C. difficile* infection 30 days before HSCT hospital admission and upon hospital discharge. Patients who experienced CDAD were followed as outpatients for 100 days posttransplantation to determine clinical outcomes, infection recurrence, and the development of GI graft-versus-host disease (GVHD). All HSCT recipients received prophylactic acyclovir, a triazole antifungal, and a fluoroquinolone antibiotic unless they were receiving alternate treatment for a prior infection. All patients received a proton pump inhibitor during hospital admission.

Allogeneic HSCT recipients (allo-HSCT) remained on the same prophylactic antimicrobial regimen throughout day +100 after HSCT, whereas autologous HSCT recipients (auto-HSCT) received acyclovir once daily upon hospital discharge. All positive bacterial and fungal cultures were used for analysis, except single coagulase-negative *Staphylococcus* cultures and vancomycin-resistant *Entereococcus faecium* (VRE) broncheoalveolar specimens. Rectal VRE surveillance cultures were performed weekly during hospital admission.

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GVHD was diagnosed by tissue biopsy in most patients (82%) and by clinical assessment and endoscopy in the remainder. Due to NMH policy changes, half the HSCT recipients received a neutropenic diet during neutropenia, whereas the other half did not. The main difference between a neutropenic and non-neutropenic diet was the allowance of fresh fruits, nuts, seeds, and vegetables.

Metronidazole 500 mg 3 times daily was given as initial therapy for all new CDAD cases. Patients who experienced persistent diarrhea or who were intolerant to metronidazole were empirically switched to oral vancomycin 125 to 250 mg 4 times daily for a minimum of 10 days or until resolution of neutropenia and discontinuation of systemic antibiotics. Antibiotic success was defined as complete abatement of CDAD symptoms or negative *C. difficile* follow-up cultures for patients with persistent diarrhea. Molecular characterization studies of *C. difficile* isolates were not available for all patients and thus were not reported.

Statistics

Bivariate analysis was performed using chi-square analysis and Fischer's *t*-test to assess the difference in variables between CDAD and non-CDAD patients. Cox regression analysis was used to identify risk factors for CDAD and to create a risk stratification model for the development of CDAD. This study was approved by the NMH Institutional Review Board.

RESULTS

Among the 822 transplantation patients reviewed were 615 autologous and 207 allogeneic transplantation patients. Diarrhea was reported in 83% of all HSCT cases. Eighty-five cases (10.3%) of CDAD were identified. CDAD was diagnosed 8 days (median) after stem cell infusion during the time when most patients were neutropenic. Five patients developed CDAD before stem cell infusion. The time to stem cell engraftment was 1 day longer for patients with CDAD. Patient demographics are shown in Table 1. No significant differences were observed for sex, diagnosis, or conditioning regimens between CDAD and non-CDAD HSCT recipients.

The results of bivariate analysis are shown in Table 2. CDAD occurred significantly more often in allogeneic patients (14.5%) compared with autologous patients (8.5%). For patients who underwent an allogeneic transplantation, the incidence of CDAD was similar between patients who received either a full myeloablative or a reduced-intensity conditioning regimen. More CDAD patients experienced febrile neutropenia and were more likely to develop a microbiologically confirmed infection. The general (non-neutropenic) diet was associated with a lower, although not statistically significant, risk for CDAD. Patients who were colonized with VRE (rectal surveillance positive) developed CDAD more often than those who were VRE negative. CDAD patients received aztreonam and vancomycin more often and were treated with ciprofloxacin and vancomycin for a longer duration of therapy.

CDAD was diagnosed before GI GVHD in 10 of 13 allo-HSCT recipients (77%). The incidence of acute grades III-IV GI GVHD was significantly higher in CDAD patients 60 and 100 days posttransplantation. Overall nonrelapse mortality was greater in allo-HSCT recipients with CDAD who developed aGVHD compared to allo-HSCT recipients who developed aGVHD without CDAD. There was no difference in overall mortality between CDAD and non-CDAD auto-HSCT recipients.

Table 1
Demographics

Characteristic	Number
Age yr (range)	58 (18-78)
Men	443
Transplantations	822
Autologous	617
Allogeneic	205
Diagnosis	
Myeloma	445
Non-Hodgkin lymphoma	152
Acute myeloid leukemia	130
Hodgkin disease	38
Acute lymphocytic leukemia	14
Myelodysplastic syndrome	8
Chronic lymphocytic leukemia	8
Other	28
Conditioning regimen	
High-dose melphalan (200 mg/m^2)	513
BEAM	173
Busulfan and fludarabine or Cytoxan	131
Reduced-intensity (MEL 100 mg/m^2 ± Cytoxan)	83
Other	22

Mel indicates Melphalan.

Table 2
Risk Factor Analysis for Clostridium difficile Infection

Characteristic	CDAD n (%)	Non-CDAD n (%)	*P* Value
HSCT recipients	85	737	
Time to CDAD post-HSCT, day (range)	8 (−4-45)		
Time to engraftment	12	11	
Male	47 (56)	434 (59)	.55
Age, yr (range)	60 (21-74)	56 (18-74)	.062
HSCT			
Allogeneic	30 (37)	176 (24)	.01
Autologous	52 (63)	566 (76)	.01
Diagnosis			
Myeloma	42 (51)	400 (54)	.642
Acute leukemias	16 (19)	126 (17)	.54
Non-Hodgkin lymphoma	10 (12)	140 (19)	.173
Conditioning regimen			
High-dose melphalan based	46 (56)	467 (63)	.337
BEAM	7 (8)	103 (14)	.23
Busulfan and fludarabine/ Cytoxan	14 (17)	117 (16)	.751
RIST (MEL 100/m^2 ± Cytoxan)	14 (17)	69 (9)	.034
Neutropenic fever	63 (74)	441 (60)	.0132
Auto-HSCT	36 (77)	315 (56)	.36
Allo-HSCT	27 (77)	126 (74)	.84
Neutropenic diet	32 (39)	377 (51)	.036
Auto-HSCT	29 (58)	281 (50)	.03
Allo-HSCT	25 (71)	71 (42)	.0015
VRE-positive surveillance cultures	31 (35)	143 (19)	.0006
Auto-HSCT	18 (33)	101 (18)	.0042
Allo-HSCT	13 (36)	42 (28)	.141
Microbiologically confirmed bacterial cultures	37 (42)	155 (21)	.0012
Auto-HSCT	16 (.32)	105 (15)	.0125
Source of infection			
Blood	24 (28)	138 (19)	.046
Urine	6 (7)	23 (3)	.04
Broncheoalveolar/sputum	9 (11)	13 (2)	.001
Antibiotics			
Ciprofloxacin	79 (93)	688 (93)	.99
Ciprofloxacin duration	10.47	8.37	.0001
Cefepime	63 (77)	500 (68)	.103
Cefepime duration	6.56	5.5	.08
Piperacillin/tazobactam	13 (16)	135 (18)	.99
Piperacillin/tazobactam duration	7.46	5.54	.28
Vancomycin	59 (72)	404 (55)	.003
Vancomycin duration	7.46	5.54	.28
Atreonam	18 (21)	99 (14)	.0058
Atreonam duration	7.71	4.93	.036
Corticosteroids	14 (17)	104 (14)	.505
Allo-HSCT	21 (60)	50 (29)	.002
Grades II-IV GI GVHD (60 days post-HSCT)	10 (28)	19 (11)	.0139
Grades II-IV GI GVHD (100 days post-HSCT)	13 (37)	22 (1)	.0024
Overall mortality (auto-HSCT)	2 (2)	16 (2)	.99
Overall mortality (allo-HSCT)	5 (6)	13 (2)	.178
Overall mortality with grades II-IV GVHD	10 (29)	17 (10)	.0104

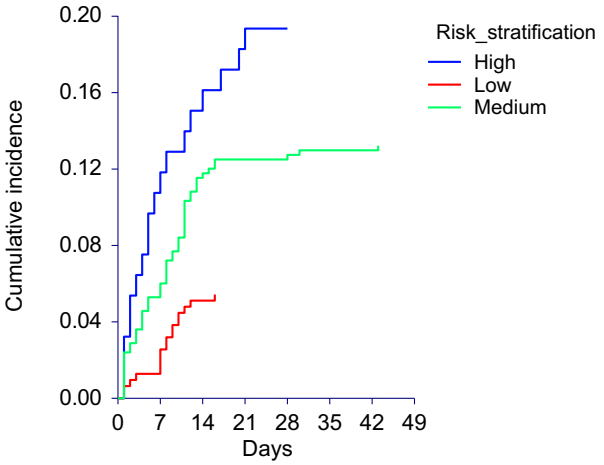

Figure 1. CDAD risk stratification. Low: Age ≤60 + no VRE colonization + autograft. Medium: Age >60 + no VRE colonization + autograft. Age ≤60 + VRE colonization or allograft. High: All others. $P < .001$.

Cox regression analysis identified age >60 years, receipt of an allo-HSCT, and VRE colonization to be independently significant factors associated with CDAD ($P < .001$). Based on the three risk factors identified by regression analysis, a risk stratification model was developed demonstrating widely disparate incidences of CDAD (Figure 1). Risk ratios were as follows: age >60, 1.4639 (range, 1.1828 to 1.8117); allo-HSCT, 1.9072 (range, 1.2174 to 2.9880); and VRE colonization, 1.6501 (range, 1.0526 to 2.5869). The incidence of CDAD was >20% in patients with all three risk factors.

Most CDAD patients (87%) were successfully treated with metronidazole 500 mg orally three times daily. Oral vancomycin was substituted in 17 patients (20%) because of intolerance to metronidazole or for persistent diarrhea despite negative follow-up *C. difficile* cultures. Clinically, *C. difficile* was eradicated from the stool in all patients as determined by repeat negative cultures in patients with persistent diarrhea or formation of a normal stool (repeat cultures not performed). CDAD recurred in 10 patients (12%) a median of 37 days (range, 7 to 92 days) after initial CDAD infection. Only one patient experienced more than one recurrence of CDAD. *C. difficile* A or B toxin was negative in 22 patients (27%) when the toxigenic *C. difficile* stool culture was positive.

The number of transplantation cases performed at NMH during the study period increased >40% (2004, 180 transplantations per year; 2008, 250 transplantations per year). The number of CDAD cases was relatively constant each month (median, 1.7 cases) over the entire 4-year study period. Predictably, a move to a new hospital facility resulted in the complete disappearance of all CDAD cases for the first 3 months after the new facility opened. Thereafter, CDAD cases increased steadily over the subsequent months to rates similar to those observed in the older facility.

DISCUSSION

CDAD is the most common cause of hospital-acquired diarrhea and has been increasing in incidence over the last 10 years. Community-acquired CDAD is emerging as a previously unrecognized entity that is frequently underdiagnosed. Antibiotic and proton pump inhibitor overuse, older age, chemotherapy, and outbreaks of a newly recognized hypervirulent *C. difficile* NAP-1 strain have been identified as causes for the increased incidence of CDAD [4-6].

The period surrounding HSCT is replete with medical (chemotherapy, antimicrobials, immunosuppression) and environmental (dietary changes and restrictions, lateral transmission of disease via health care workers) factors that predispose HSCT recipients to diarrhea. Diarrhea is almost universally observed after stem cell transplantation. *C. difficile* has been recognized as a cause of diarrhea in HSCT recipients for many years.

The overall incidence of CDAD during our 4-year study period was 10.3%. CDAD rates within our institution have increased from 6.4%, as previously reported in 2000, to 8.5% over the past 10 years after autologous transplantation. CDAD rates were significantly higher in allogeneic recipients. Table 3 summarizes retrospective studies conducted from HSCT centers since 2000. For most studies, patient accrual was completed more than 10 years ago. Variable CDAD incidence rates, generally between 1% to 12% for auto- and allo-HSCT recipients, were observed [7-13]. The most recently published epidemiologic study reported by Chopra et al. [13], conducted during the same time period as the present study, observed CDAD rates similar to our results. The incidence of CDAD was reported to be several times greater than the general population and significantly higher than patients treated for cancer. Collectively, the most current studies report higher CDAD rates compared with earlier studies and suggest CDAD rates are increasing within the stem cell population, paralleling the rise in CDAD observed within the general population.

A limitation of previously reported CDAD studies conducted within the HSCT population has been the inability to identify unique risk factors associated with the development of CDAD, perhaps due to small sample sizes. The present study identified 3 risk factors associated with the development of CDAD by multivariate regression analysis.

Age >60 years was identified as a risk factor for CDAD after HSCT. This may be a direct result of the increasing number of patients > 60 years old undergoing autologous and allogeneic transplantations over the past decade. Data from the Center for International Blood and Marrow Transplant Research [14]

Table 3
CDAD Studies in HSCT Recipients from 2000-Present

Author (year)	Study Period	Transplant Type	Number	Incidence of CDAD (%)	Risk Factors Identified
Chakrabarti [7] (2000)	1994-99	Allogeneic	75	13	>High-grade GVHD, nonrelapse mortality
Avery [8] (2000)	na	Autologous	80	5	None
Van Kraaj [9] (2000)	1995-96	Autologous/allogeneic	47	Auto = 4 Allo = 0	None
Tomblyn [10] (2002)	1998-2000	Autologous/allogeneic	119	Auto = 7 Allo = 0	None
Jillella [11] (2003)	1997-2001	Autologous	54	6.7	None
Arango [12] (2006)	1996-2001	Autologous	242	15.5	Antibiotics
Chopra [13] (2011)	2005-6	Autologous/allogeneic	361	Auto = 8 Allo = 18	None

reported the number of allogeneic transplantations performed in patients aged >60 years has more than doubled between 1993 to 2003 and 2004 to 2008. Autologous transplantations have also increased significantly [14]. Data from discharged hospital patients within the nontransplantation setting have also reported a several fold higher age-adjusted rate of clostridium difficile infection among persons older than 64 years [15].

Many comorbid conditions increase with age, and these conditions increase the likelihood for altered chemotherapy distribution, metabolism, and excretion, leading to increased toxicity. For many conditioning regimens, mucositis along the entire GI tract is the dose-limiting toxicity. Chemotherapeutic agents alone have been shown to be independent risk factors for the development of clostridium difficile infection. Changes to the intestinal microbiota after chemotherapy alone are similar to those observed in CDAD patients after antibiotic administration. These changes have been associated with increasing inflammation and permeability and reduction in the protective mucosal layer, epithelial repair, and immune responsiveness. The role of excessive damage to the GI tract caused by chemotherapy and its association with infection in older patients requires further investigation [16-22].

Receipt of an allogeneic transplantation was observed to be an independent risk factor for the development of CDAD. A number of factors could be responsible for the pathogenesis of CDAD in this population. Allo-HSCT recipients typically experience a longer period of neutropenia before stem cell engraftment, increasing the exposure to broad-spectrum antibiotics, which are known risk factors for the development of CDAD. Additionally, longer hospital admissions place patients at an increased risk for transmission of nosocomial infection.

Patients receiving immunosuppressants are at increased risk for developing infections. Some evidence suggests these agents may be directly linked to CDAD. Experimentally, in mice not exposed to antibiotics, cyclosporine administration was shown to lead to the development of mild to moderate CDAD [23]. Clinically, a retrospective review of liver transplantation recipients who received immunosuppressant agents similar to those used during HSCT, yet typically receiving significantly less antibiotic use, reported CDAD rates similar to our study, indirectly suggesting a possible link between immunosuppression and CDAD [24].

We identified VRE rectal colonization as a newly recognized risk factor for the development of CDAD in HSCT recipients. VRE colonization rates were similar among allogeneic and autologous recipients. Recent laboratory and clinical studies have suggested a link between VRE and *C. difficile* colonization and infection. Experimentally, a murine model has shown VRE cultures administered exogenously after the administration of antibiotics almost completely displaced the normal microbiota of the intestines, allowing for the growth of antibiotic-resistant microbes and *Clostridium* ssp. This effect lasted for several weeks. The authors also described similar dramatic changes to the intestinal microbiota in two allo-HSCT recipients associated with endogenous intestinal VRE stool culture predominance. These changes were not seen in three control patients who did not develop VRE bacteremia [25].

Additional evidence for a link between VRE colonization or infection and *C. difficile* infection was reported in a small cohort study (n = 10) of patients with acute leukemia. The correlation between CDAD and VRE was observed in the reverse order of our findings: *C. difficile* infection and treatment increased the likelihood of VRE infection. All patients in that study had prior VRE colonization [26]. Comparative retrospective trials from patients within the general population from acute care facilities or nursing homes have also identified a relationship between VRE infection or colonization and coinfection with *C. difficile*, both associated with antibiotic administration [27,28]. Finally, the results of our prior study, which questioned the role of a low microbial diet during HSCT, identified a higher incidence of *C. difficile* infection in patients and positive VRE surveillance cultures [29]. Collectively, the data suggest VRE colonization or infection and *C. difficile* infection together occur more frequently than previously recognized.

An association between ciprofloxacin, vancomycin, and aztreonam use and duration of therapy and the development of CDAD was observed. Our results are consistent with previously reported results of antibiotic-associated CDAD from studies both within the general population and in HSCT recipients. Antibiotic-associated CDAD has long been implicated with virtually all antibiotics, although differences in associated incidence rates exist that depend on the spectrum of activity and pharmacokinetic properties [30]. Attempting to reduce antibiotic-induced CDAD through restriction of fluoroquinolone antibiotic prophylaxis during HSCT is problematic, as recent evidence-based guidelines state flouroquinolone prophylaxis be considered for high-risk patients with expected durations of prolonged and profound neutropenia [31]. The risk for developing CDAD appears to be even greater with the concomitant use of antibiotics and proton pump inhibitors [32].

Our results show CDAD patients are more likely to develop grades II-IV GI GVHD both 60 and 100 days post-HSCT. These results are consistent with a published case-control (37 CDAD patients and 67 control subjects) study by Dubberke et al. [33] that reported a significant increase in new-onset GVHD, new-onset severe GVHD, and new-onset gut GVHD associated with CDAD 180 days post-HCST. Also observed were significantly higher rates of bloodstream infections after diagnosis with CDAD and increased nonrelapse mortality rates in CDAD patients [33]. The results establish CDAD as one of the many causes associated with morbidity and nonrelapse mortality in HSCT recipients.

Despite a 40% increase in the number of HSCT transplantations performed each year over the 4-year study period, the monthly CDAD rate remained constant throughout the study period. Patient isolation, glove and gown precautions, and handwashing with soap were maintained equally throughout the study period. *C. difficile* is a transmissible nosocomial pathogen, and one could speculate that heightened awareness and improved infection control might have been responsible for curtailing horizontal transmission of CDAD. Implementation of an infection control program is essential for preventing transmission of CDAD.

Most CDAD cases completely resolved with metronidazole treatment. Patients who were empirically deemed treatment failures or intolerant to metronidazole were successfully salvaged with oral vancomycin treatment. CDAD recurrence was low and similar to recurrence rates reported from studies published within the general population. Published evidence-based guidelines from the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America recommend oral vancomycin therapy as initial treatment for patients at high risk (age >64 years, elevated white blood cell count and serum creatinine levels) for the

development of moderate to severe CDAD [34]. Respondents to a recent survey of transplantation centers (n = 34) regarding initial drug therapy for the treatment of CDAD indicated most institutions (74%) still prescribe metronidazole as front-line drug therapy (personal communication, Steven Trifilio, Northwestern Memorial Hospital, December 20, 2011). Prospective studies are needed to determine whether the unique serious GI toxicities encountered during stem cell transplantation warrant similar antibiotic recommendations.

The rate of false-negative C. *difficile* toxin A or B assay results demonstrate a lack of sensitivity for this assay and the need to confirm C. *difficile* in stool culture, especially in patients with persistent diarrhea. We believe the development of the risk stratification model provided in Figure 1 will assist practitioners in critical clinical decision-making pathways. Patients at highest risk for the development of CDAD may benefit from increased VRE surveillance, minimizing antibiotics known to promote the development of CDAD, and heightened environmental and isolation precautions that may reduce horizontal transmission of CDAD.

With an overall incidence >10%, CDAD is a significant source of infectious complications within the HSCT setting. Based on patient characteristics and interventions, the risk ranges from 6% to 20%. Identification and correction of reversible risk factors could potentially reduce the incidence of CDAD.

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